Application, characterization and model development of *Cassia obtusifolia* seed gum in treating raw and complex industrial effluent together with alum

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ABSTRACT

Coagulation is effective, simple to operate, and widely used in treatment of industrial effluent. However, commonly used inorganic coagulants pose detrimental effects on human health and living organisms, and generate large amount of toxic sludge. Thus, this study investigated the optimized use of *Cassia obtusifolia* alone or combination of alum with lower dosages in treatment of industrial effluent. In this study, raw pulp and paper mill effluent was used as a model wastewater in confirming the potential use of *C. obtusifolia* seed gum as a plant0based coagulant or coagulant aid. Response surface methodology enabled the study of interactive effects between operating treatment conditions, and to determine the optimal treatment conditions. The analysis of variance results obtained in the present study reflected significant quadratic models and strong correlations between operating conditions and treatment performance. In addition, current findings suggested that the coagulation mechanism using *C. obtusifolia* seed gum was most likely based on adsorption with inter-particle bridging. Floc characterization also showed distinctive results, indicating the presence of active functional groups and the thermal stability of floc formed using *C. obtusifolia* seed gum. A significant reduction of coagulant dosages up to 67-91% was attained with the combined use of *C. obtusifolia* seed gum and alum. At natural pH of raw PPME (pH 7.2), optimal dosages of 0.17 and 0.09 g/L *C. obtusifolia* seed gum and alum, respectively were required to achieve maximum total suspended solids and chemical oxygen demand removals (91.6 and 58.3%, respectively). The present study revealed *C. obtusifolia* seed gum could be used as a potential plant-based coagulant or coagulant aid in treatment of raw and complex industrial effluent.

Keywords: Optimization; Natural coagulant; Response surface methodology; Coagulation; Alum; Wastewater treatment

1. Introduction

Solid-liquid separation through coagulation process is considered as an important process in wastewater primary treatment (Lee et al., 2012). It is effective, simple to operate, and widely used in wastewater treatment (Wang et al., 2014). Inorganic coagulants are commonly used in coagulation process. However, the drawbacks associated with their prolong and excessive usage include detrimental effects on human health and living organisms, ineffectiveness in low-temperature water, high procurement cost, and significant generation of toxic sludge (Antov et al., 2012; Yang et al., 2013).

On the contrary, natural coagulants are favorable due to its biodegradability, low toxicity, low cost and low sludge production (Antov et al., 2012). *Cassia obtusifolia* is a plant that grows 1.5-2.5 m in height, and bears 8000 seeds per plant (Mackey et al., 1997). Department of Agriculture, Fisheries and Forestry, Queensland, Australia (2014), reported that 2000 seeds/m^2 of soil is harvested from local seed reserves. The present work was considered as a notable advancement in introducing the use of emerging plant-based coagulant, *C. obtusifolia*, in real wastewater treatment. Thus far, *C. obtusifolia* is explored mostly in the field of medicine and bioscience, and not many works are associated with real wastewater treatment processes (Vadivel et al., 2011; Queiroz et al., 2012; Sultana et al., 2012; Cai and Chen, 2013).

Among many factors, type of coagulant, coagulant dosage, effluent pH, mixing, temperature, and retention time can influence the coagulation process (Wang et al., 2014). Therefore, optimization of these factors is crucial in increasing the coagulation treatment efficiency. Conventional one-factor-at-a-time approach is carried out by varying a single factor while keeping all other factors in constant. However, this approach is time consuming, laborious, and expensive because large number of experiments must be carried out (Wu et al., 2009; Teh et al., 2014a). Thus, response surface methodology (RSM) was proposed in this study. RSM is a combination of mathematical and statistical techniques for designing experiments, building models, evaluating the effects of several factors and their interactions, and achieving optimal conditions for desirable responses with a limited number of planned experiments (Srinu Naik and Pydi Setty, 2014; Wang et al., 2014). Recently, this optimization technique has been applied in a wide range of processes, such as absorption (Bhanarkar et al., 2014), engine performance (Sivaramakrishnan and Ravikumar, 2014), biosorption (Aytar et al., 2014), electrocoagulation (Sridhar et al., 2014), biodiesel production (Moradi and Mohammadi, 2014) and others. However, to the best of our knowledge, the application of RSM in optimizing the combined use of natural coagulant and inorganic coagulant in pulp and paper mill effluent (PPME) treatment is not yet reported. Generally, appropriate combination between natural and inorganic coagulants in water treatment is promising in terms of both treatment efficiency and cost (Teh and Wu, 2014; Teh et al., 2014a).

The main objective of the present work was to conduct a comprehensive optimization study on an emerging natural coagulant, *C. obtusifolia* seed gum, in treatment of raw and complex industrial PPME. To reduce the dosage of coagulants in PPME treatment, optimization study was also conducted in investigating the potential combination between alum and *C. obtusifolia* seed gum. A model relating the coagulation performance (in terms of both removals of total suspended solids and chemical oxygen demand) to the key operating parameters (coagulant dosage, effluent pH, and slow-mixing duration) was developed using RSM approach. In addition, better insights into coagulation mechanism and detailed floc of combination between alum and *C. obtusifolia* seed gum characterization were attained using a scanning electron microscope (SEM), Fourier-transform infrared spectroscopy (FTIR), and thermogravimetric analysis (TGA).

2. Materials and Methods

2.1 Materials

Raw PPME was collected from Muda Paper Mills, Selangor, that generates up to 25000 m^3/day of wastewater. The average pH, total suspended solids (TSS), and chemical oxygen demand (COD) of the collected PPME was 7.2, 841 mg/L, and 1453 mg/L, respectively. The collected PPME was stored at 4 °C to reduce possible biodegradation. The raw PPME was used in jar-test experiments without introducing any dilution.

C. obtusifolia whole seeds were purchased locally (Fig. 1). The seeds were ground into finer granules using Pulverisette 14 Variable Speed Rotor Mill (Germany) and were kept in an air-tight glass container. Fresh *C. obtusifolia* seed gum stock solution of 25 g/L was prepared daily. Alum (aluminium sulfate octadecahydrate) was purchased from Sigma-Aldrich with A.C.S grade and used as a control without further purification.

2.2 Experimental procedure

A jar-test procedure was set up at room temperature for each experimental run. Each beaker contained 300 mL of raw and undiluted PPME. The effects of initial effluent pH, coagulant dosage, and slow-mixing time were investigated by using *C. obtusifolia* seed gum and alum separately. On the other hand, similar effects were studied for combination use of both *C. obtusifolia* seed gum and alum but at natural pH of raw PPME.

The initial pH (pH 7.2) of the PPME was adjusted accordingly (pH 1.98-7.02) using 1 mol/L of HCl or NaOH when required. Coagulants (0.01-3.57 g/L) were added into the PPME during flash-mixing stage (150 rpm for 5 minutes). The effluent was subsequently subjected to slow-mixing at 10 rpm. The floc was allowed to settle for 3 min. The sample for analysis was taken 2 cm below the surface level for measurement of final TSS and COD values. Each run was repeated in 3 replicates (n=3). The TSS and COD removals were calculated as shown in equations (1) and (2), respectively:

$$\text{TSS removal, \%} = \frac{\text{TSS}_i - \text{TSS}_f}{\text{TSS}_i} \times 100\% \quad (1)$$

$$\text{COD removal, \%} = \frac{\text{COD}_i - \text{COD}_f}{\text{COD}_i} \times 100\% \quad (2)$$

where TSS_i and TSS_f are initial and final TSS values (mg/L), respectively, and COD_i and COD_f are initial and final COD values (mg/L), respectively.

2.3 Experimental design and model development

Central composite design (CCD) was selected as the standard RSM for the optimization of key operating factors (coagulant dosage, initial pH, and slow-mixing time) in the present study. In this study, CCD was recognised as an efficient design tool to fit second-order models. The range and coded levels of the selected operating factors (independent variables) for model development are shown in Table 1. Treatment efficiency in terms of TSS and COD removals were chosen as the responses (dependent output variables). Design-Expert® (Version 6.0.10, Stat-Ease Inc. Minneapolis, USA) was used to fit the responses using a predictive polynomial quadratic model to obtain a correlation between the responses and factors (Teh et al., 2014a). A general form of second-order polynomial quadratic model used to describe the effects of different factors on the responses was represented in equation (3) (Hay et al., 2012):

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i<j} \beta_{ii} X_i X_j \qquad (3)$$

where Y = responses (TSS and COD removals); β_0 = constant; β_i = linear coefficient; β_{ii} = quadratic coefficient; β_{ij} = interactive coefficient; X_i and X_i = actual factor level. The interactive effects between the operating factors on the responses were represented as 3-D contour plots. In addition, the TSS and COD removals were maximized to achieve the highest coagulation performance possible for each coagulant (*C. obtusifolia*, alum, and combined use of both coagulants) at optimal values of the operating factors. Experimental runs were then conducted in triplicate to serve as verification of the obtained optimal treatment conditions from the developed quadratic models through equation (3).

2.4 Floc characterization

Samples of the coagulant floc were analysed using special instrumentation. SEM (Hitachi S3400N-II model, Japan) was used to analyze the morphology of floc. The infrared spectra of floc was analysed using a FTIR spectrometer (Thermo Scientific Nicolet iS10, USA) from 400 to 4000 cm^{-1}. Lastly, TGA of floc was determined using a thermal analyzer (TA Instrument TGA Q50, USA) under nitrogen atmosphere with a heating rate of 10 °C/min to 800 °C.

3. Results and Discussion

3.1 Assessment of experimental results through RSM

Twenty experimental runs, including center and axial points were investigated in CCD-designed experiment to visualize the effects of each operating factor on the responses. Based on the experimental results, quadratic models were recommended to represent the coagulation performance of each coagulant used in this study. Table 2 presents the Analysis of variance (ANOVA) results of the regression parameters from the predicted quadratic models in Table 3. The p value is used to estimate whether F value is large enough to indicate statistical significance (Sridhar et al., 2014). Values of p less than 0.05 imply that the quadratic model terms are significant whereas values greater than 0.10 indicate that the model terms are insignificant. All quadratic models predicted in the present work were significant as indicated by the p values less than 0.0001 (Table 2). Adequate precision ratios obtained were greater than 4 (Table 2), indicating that adequate signal for all models could be used to navigate the design space (Bhanarkar et al., 2014).

The lack-of-fit test describes the variation of the data around the fitted model (Muhamad et al., 2013). If the quadratic models fit the data, the lack-of-fit test will be insignificant. All models have insignificant lack of fit values (Table 2), indicating a significant model correlation between the operating factors and responses (Muhamad et al., 2013). Table 2 shows that a good quadratic fit was achieved for all models whereby, all R^2 values were close to 1, denoting a satisfactory adjustment of the quadratic models to the experimental data. It should be noted that a R^2 value greater than 0.75 indicates the aptness of the model (Srinu Naik and Pydi Setty, 2014). Moreover, a closely high value of the adjusted R^2 indicates the capability of the developed model to satisfactorily describe the system behaviour within the range of operating factors (Bhanarkar et al., 2014). In terms of actual factors, quadratic models in reduced form of both TSS and COD removals are expressed in Table 3.

3.2 Optimization of operating treatment conditions

Optimization of the treatment process using *C. obtusifolia* seed gum and/or alum was conducted using RSM to minimize the operating cost by reducing the coagulant dosages but achieving the highest TSS and COD removals from the wastewater. The highest removal efficiencies were maximized using the optimal treatment conditions and the results are presented in Table 4. Under the optimum conditions, the experimental TSS and COD removals were very close to the predicted values, in which case, the error obtained were in the range of 0-2.2 and 0.4-6.1%, respectively.

Overall, natural and unmodified *C. obtusifolia* seed gum demonstrated a positive and comparatively high in both TSS and COD removals (Table 4). A slight difference in TSS removal was noted between the use of *C. obtusifolia* seed gum and alum. However, the COD removal using *C. obtusifolia* seed gum alone was 29.2 and 27.4% lower than alum and the combined use of both coagulants, respectively. Since *C. obtusifolia* seed gum is an organic plant-based compound, an addition of *C. obtusifolia* seed gum into coagulation process would contribute to the increase of dissolved organic matter in the treated effluent, resulting in higher final COD value (Teh and Wu, 2014; Teh et al., 2014a). However, the combined use between *C. obtusifolia* seed gum and alum at natural pH of wastewater substantially reduced the optimal dosage by 90.5 and 62.5%, respectively as compared to the use of *C. obtusifolia* seed gum or alum alone (Table 4). Moreover, the combination of both coagulants resulted in TSS and COD removals comparable to alum. Based on these findings, natural *C. obtusifolia* seed gum was proven to be an effective natural coagulant in treating raw and complex industrial effluent

(Subramonian et al., 2014). Furthermore, *C. obtusifolia* seed gum could be used as a coagulant aid by reducing the amount of alum required in coagulation process but achieving almost similar treatment efficiency when higher dosage of alum was used.

3.3 Effect of operating parameters on treatment efficiency

Optimization study using *C. obtusifolia* seed gum alone revealed that the increase in seed gum dosage beyond optimal dosage (1.79 g/L) improved the TSS and COD removals due to re-stabilization of suspended particles at higher dosages. *C. obtusifolia* seed gum also showed higher removals at acidic pH due to better adsorption of hydrolysed organic pollutants (Subramonian et al., 2014). Based on the optimization study of alum alone, an increase in alum dosage beyond optimal value (0.24 g/L) showed insignificant improvement in removals because of charge reversal might occur when aluminium species were present in excess (Jangkorn et al. 2011; Teh et al., 2014a). On the other hand, alum performed better at pH 6 due to better adsorption of colloids onto its surface at pH near to neutral (Subramonian et al., 2014).

Since alum demonstrated better efficiency under natural pH, the optimization study using both *C. obtusifolia* seed gum and alum was conducted under the aforementioned condition (Fig. 2), with optimized conditions shown in Table 4. Generally, all response surfaces (Fig. 2) were convex in nature, suggesting that there was a well-defined optimum point for maximum TSS and COD removals (Wu et al., 2009; Tan et al., 2013). By not adjusting the initial pH of wastewater prior to treatment was not only economical but environmentally less toxic. When alum was used as a coagulant, *C. obtusifolia* seed gum behaved as a coagulant aid in bridging the coagulated particles (Bolto and Gregory, 2007). This phenomenon justified the high treatment efficiency of raw PPME (91.6 and 58.3% of TSS and COD removals, respectively) despite the large reduction in *C. obtusifolia* seed gum and alum dosages (dosage reductions of 90.5 and 62.5% of seed gum and alum, respectively) when they were used in combination (Table 4). TSS and COD removals were enhanced at longer mixing duration (Figs. 2b-c and 2e-f) up to optimum points because slow-mixing promoted better adsorption and floc formation (Kumar et al., 2011; Zhang et al., 2013). After optimum points, the improvements of TSS and COD removals seemed to be less significant. However, the removals decreased at higher mixing time for both types of coagulants due to the re-dispersion and re-stabilization of floc. This phenomenon was also observed by Şenġil (1994) when alunite ore was used as a coagulant aid.

3.4 Floc morphology

Evaluation of floc morphology provided a better understanding in the possible coagulation mechanism using *C. obtusifolia* seed gum. Fig. 3 illustrates SEM images of floc formed using different coagulants. Fig. 3a shows that the formation of floc formed using *C. obtusifolia* seed gum alone had high fibrous-like morphology (Shak and Wu, 2014). This phenomenon strengthened the possibility that the proposed coagulation mechanism of *C. obtusifolia* seed gum involved adsorption with inter-particle bridging. On the contrary, alum floc exhibited rough and irregular surface (Fig. 3b), which was due to aggregation of pollutants in the wastewater, as observed by Teh et al. (2014b). According to Ni et al. (2012), the irregular morphology was also attributed to the aluminum species present in the floc. The combined use of both coagulants resulted in a composite morphology, consisting of irregular surface and fibrous-like structure (Fig. 3c). This phenomenon indicates the functionality of both mechanisms (adsorption with inter-particle bridging and charge neutralization) when both *C. obtusifolia* seed gum and alum were used during coagulation of PPME.

3.5 FTIR analysis

The presence of active functional groups in *C. obtusifolia* seed gum and alum were analyzed using FTIR. The infrared spectra of floc formation using both *C. obtusifolia* seed gum and alum is shown in Fig. 4. The OH band is clearly indicated as the broad and strong bands at 3319 cm^{-1} (Singh et al., 2012). Similarly, peak at 2908 cm^{-1} was due to C-H linkages (Singh et al., 2007). The weaker bands at 1409 cm^{-1} represented the bending vibration of CH_3 and the scissor vibration of CH_2 (Ni et al., 2012). Peak positions around the region of 1010-1027 cm^{-1} were due to HOO matrix, OH stretching and C-O stretching (Singh et al., 2007; Singh et al., 2012). Other than that, peak at 1628 cm^{-1} were assigned to aluminum composite formed with the suspended particles (Singh et al., 2012). Peak at 2162 cm^{-1} was attributed to the formation of interactions between alum and the suspended particles after binding (Singh et al., 2007). A close comparison of floc spectra obtained from Subramonian et al. (2014) showed no signs of additional new peaks for the combined use of *C. obtusifolia* seed gum with alum. The absence of new peaks might confirm that no new complex was formed.

3.6 TGA

TGA provided the thermal decomposition of carbonaceous materials and thermal stabilities of the floc with rising temperature (Subramonian et al., 2014). According to Fig. 5a, a weight loss of 10% at temperature below 200 °C was a result from moisture and volatile compounds lost in all samples (Shak and Wu, 2014). As the temperature elevated from 200 to 800 °C, significant amount of weight loss (55%) was observed due to gradual decomposition of the sample. Differential thermal gravimetric (DTG) curve represented the decomposition stages for the floc (Fig.5b). Four distinctive and similar mass change regions were noted. At temperature less than 200 °C, the intra- and inter-molecular moisture were evaporated from the floc (Lee et al., 2012). On the other hand, the sudden loss of mass observed after 200°C was attributed to the loss of absorbed species. At temperatures beyond 300°C, a significant weight loss occurred due to decomposition of organic components in the floc. Severe decomposition occurred at temperatures above 600°C. The present result was concurrent with Lee et al. (2012), reporting that most components in the floc decomposed at high temperatures. The weightlessness at high temperatures (> 750 °C) was due to the total decomposition of floc (Subramonian et al., 2014).

Conclusion

Since the study of *C. obtusifolia* seed gum, especially successful combination with alum in wastewater treatment remains limited, a comprehensive study on its application in coagulation process and floc characterization was conducted. The floc characteristics using both *C. obtusifolia* seed gum with alum, revealed distinctive results on the presence of functional groups and thermal stability of the floc. Based on the combined floc morphology, possible coagulation mechanisms included adsorption with inter-particle bridging and charge neutralization. On the other hand, RSM approach served as a functional tool in investigating the optimal treatment conditions and the interactive effects between operating factors on the coagulation performance. Based on the current findings, the combined use of *C. obtusifolia* seed gum and alum tremendously reduced the optimal amount of coagulants by 90.5 and 62.5%, respectively. Without adjusting the initial pH of raw PPME, optimal dosages of 0.17 g/L *C. obtusifolia* seed gum and 0.09 g/L alum were required to achieve the maximum treatment performance (91.6 and 58.3% TSS and COD removals, respectively). The present study successfully established that *C. obtusifolia* seed gum, as an emerging plant-based coagulant, could be potentially used as a coagulant substitute or coagulant aid together with alum in treatment of raw and complex industrial effluent.

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Fig. 1. Whole seeds of *C. obtusifolia*.

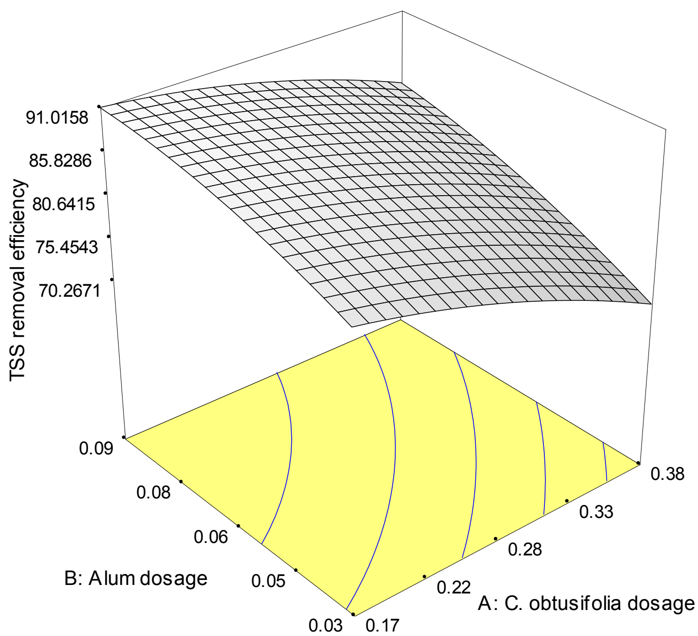

Fig. 2 (a)

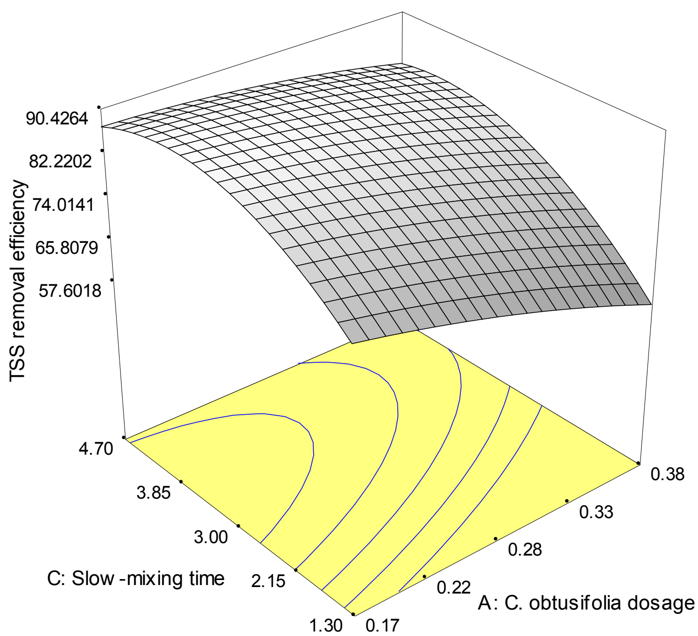
90.4264
82.2202
74.0141
65.8079
57.6018
TSS removal efficiency
4.70
3.85
3.00
2.15
1.30
C: Slow -mixing time
0.17
0.22
0.28
0.33
0.38
A: C. obtusifolia dosage

Fig. 2 (b)

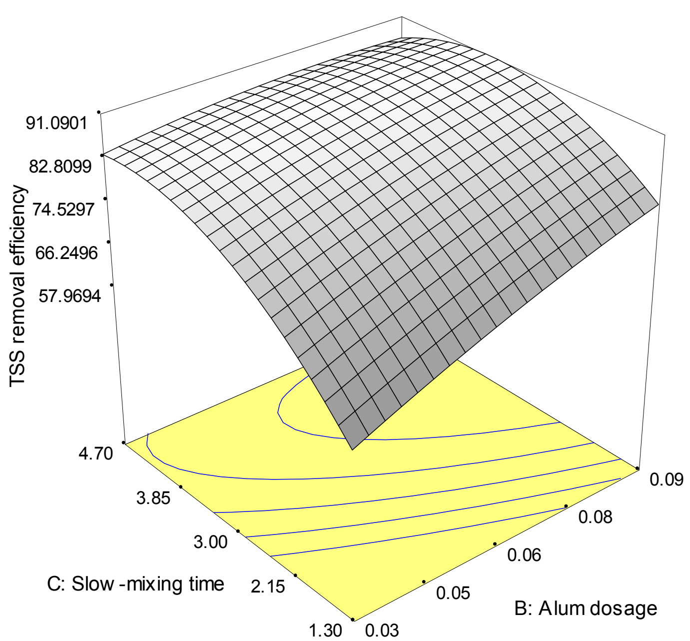
91.0901
82.8099
74.5297
66.2496
57.9694
TSS removal efficiency
4.70
3.85
3.00
2.15
1.30
C: Slow -mixing time
0.03
0.05
0.06
0.08
0.09
B: Alum dosage

Fig. 2 (c)

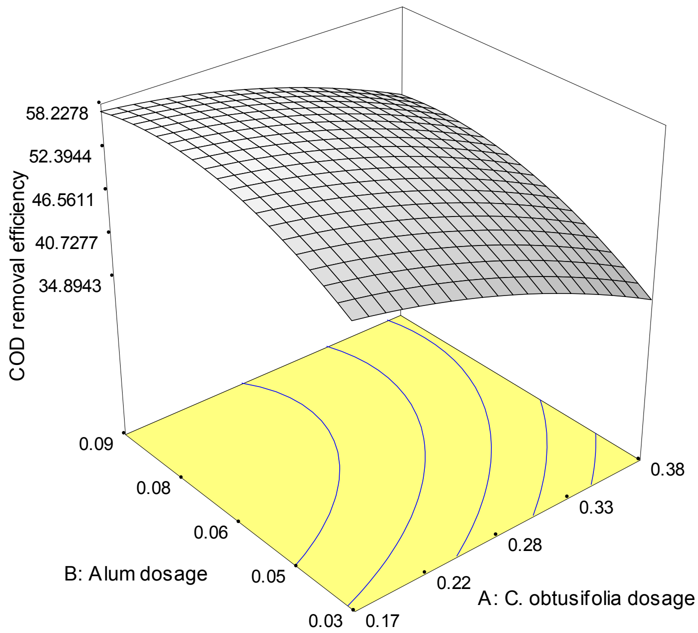
58.2278
52.3944
46.5611
40.7277
34.8943
COD removal efficiency
0.09
0.08
0.06
0.05
0.03
B: Alum dosage
0.17
0.22
0.28
0.33
0.38
A: C. obtusifolia dosage

Fig. 2 (d)

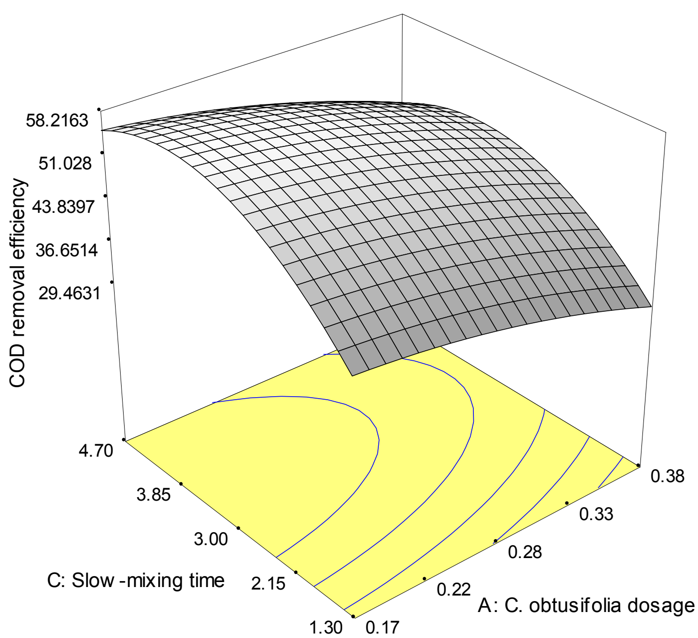
58.2163
51.028
43.8397
36.6514
29.4631
COD removal efficiency
4.70
3.85
3.00
2.15
1.30
C: Slow -mixing time
0.17
0.22
0.28
0.33
0.38
A: C. obtusifolia dosage

Fig. 2 (e)

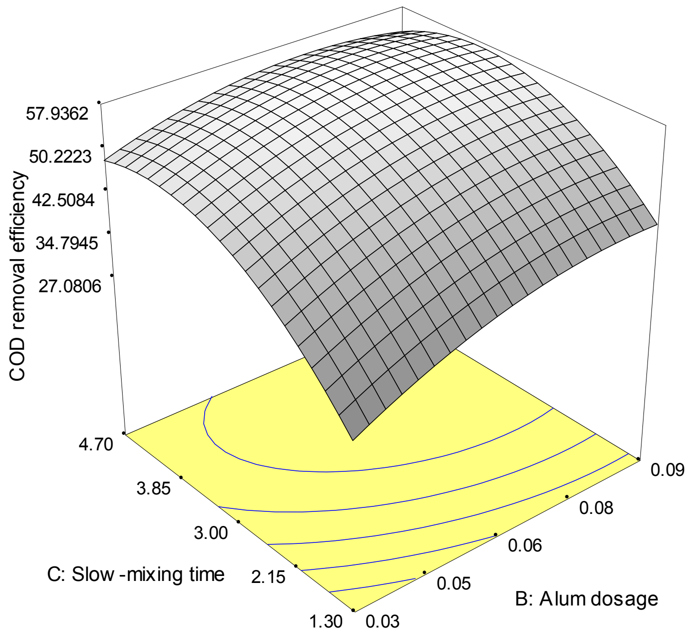

Fig. 2 (f)

Fig. 2. 3-D response surface plots showing combined use between *C. obtusifolia* seed gum and alum in TSS removal as a function of (a) *C. obtusifolia* seed gum and alum dosages, (b) *C. obtusifolia* seed gum dosage and slow-mixing time, (c) alum dosage and slow-mixing time; COD removal as a function of (d) *C. obtusifolia* seed gum and alum dosages, (e) *C. obtusifolia* seed gum dosage and slow-mixing time, (f) alum dosage and slow-mixing time. The other factors were held constant at their corresponding centre points and all combined use were done at natural pH of PPME.

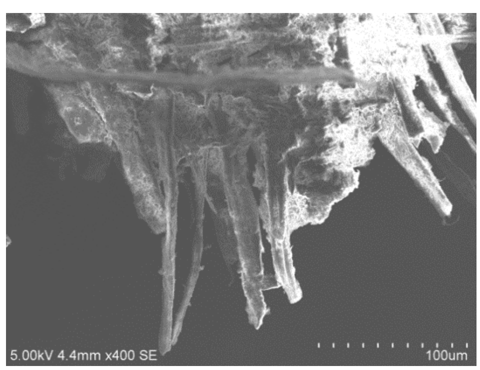

Fig. 3 (a)

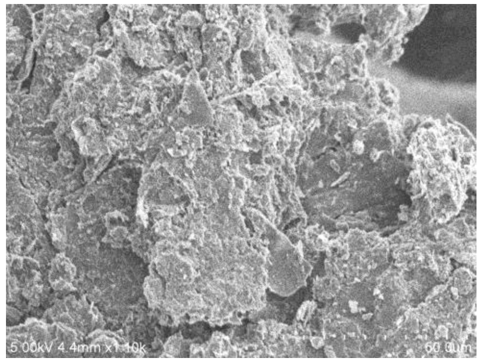

Fig. 3 (b)

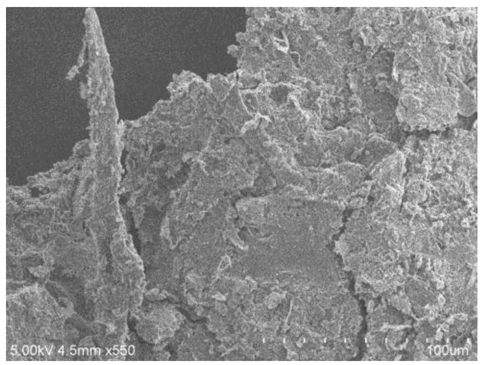

Fig. 3 (c)

Fig. 3. SEM images of floc formed using (a) *C. obtusifolia* seed gum; (b) alum; (c) *C. obtusifolia* seed gum with alum.

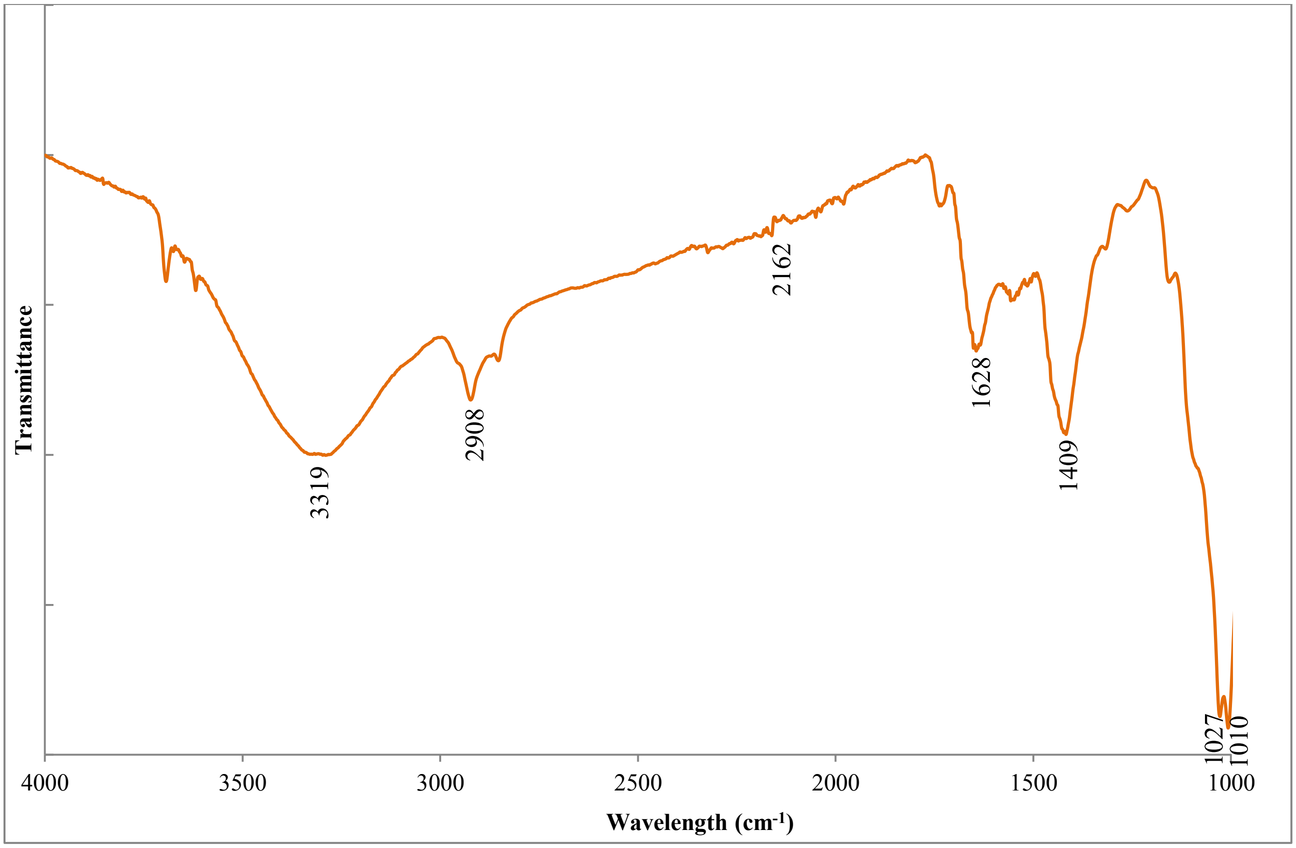

Fig. 4. FTIR spectra of floc formed using combined use of *C. obtusifolia* seed gum with alum.

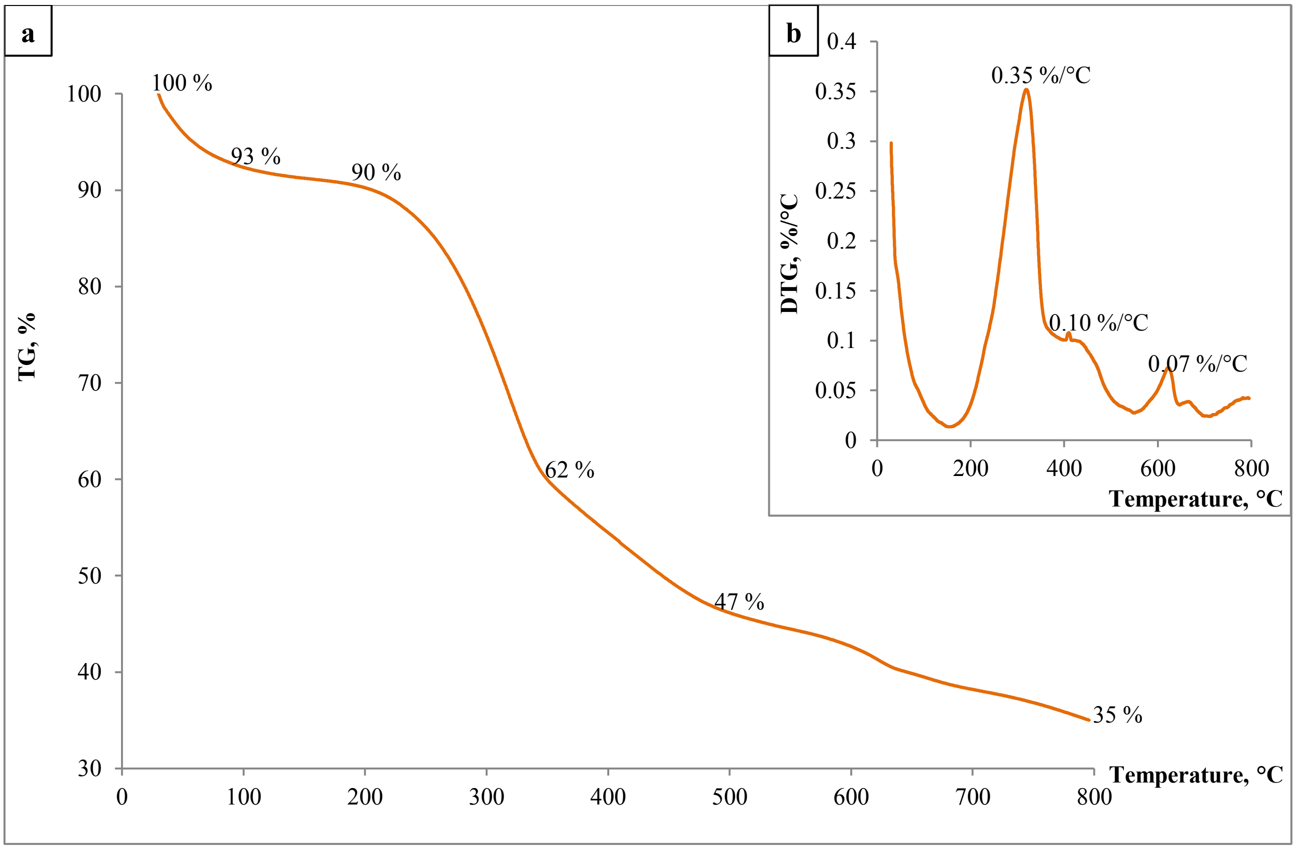

Fig. 5. (a) TG plot; (b) DTG plot of floc formed using combined use of *C. obtusifolia* seed gum with alum.

Table 1

Experimental range and coded levels of coagulant dosage, initial effluent pH, and slow-mixing time using *C. obtusifolia* seed gum and/or alum.

Factors	Symbols		Coded levels				
	Actual	Coded	-1.682	-1	0	+1	+1.682
C. obtusifolia seed gum							
coagulant dosage, g/L	X_1	x_1	0.03	0.75	1.80	2.85	3.57
initial pH	X_2	x_2	2.45	3.00	3.85	4.60	5.15
slow-mixing time, min	X_3	x_3	0.12	2.10	5.00	7.90	9.88
Alum							
coagulant dosage, g/L	Y_1	y_1	0.00	0.06	0.15	0.24	0.30
initial pH	Y_2	y_2	1.98	3.00	4.50	6.00	7.02
slow-mixing time, min	Y_3	y_3	0.14	1.30	3.00	4.70	5.86
C. obtusifolia seed gum and alum							
C. obtusifolia seed gum dosage, g/L	Z_1	z_1	0.10	0.17	0.28	0.38	0.45
Alum dosage, g/L	Z_2	z_2	0.01	0.03	0.06	0.09	0.12
slow-mixing time, min	Z_3	z_3	0.14	1.30	3.00	4.70	5.86

Table 2

ANOVA of the quadratic models developed for TSS and COD removals using *C. obtusifolia* seed gum and/or alum.

TSS removal						COD removal					
Source	Sum of squares	Degree of freedom	Mean square	*F* value	*p* value	Source	Sum of squares	Degree of freedom	Mean square	*F* value	*p* value
<u>*C. obtusifolia* seed gum</u>											
Model	11718.57	9	1302.06	13.19	< 0.0001*	Model	2581.23	9	286.80	39.43	< 0.0001*
x_1	1060.80	1	1060.80	10.75	0.0083*	x_1	121.86	1	121.86	16.75	0.0022*
x_2	5284.13	1	5284.13	53.53	< 0.0001*	x_2	1283.22	1	1283.22	176.40	< 0.0001*
x_3	2944.89	1	2944.89	29.83	0.0003*	x_3	563.12	1	563.12	77.41	< 0.0001*
x_1^2	994.76	1	994.76	10.08	0.0099*	x_1^2	134.08	1	134.08	18.43	0.0016*
x_2^2	54.48	1	54.48	0.55	0.4747	x_2^2	59.00	1	59.00	8.11	0.0173*
x_3^2	490.39	1	490.39	4.97	0.0499*	x_3^2	17.62	1	17.62	2.42	0.1507
$x_1\ x_2$	528.13	1	528.13	5.35	0.0433*	$x_1\ x_2$	154.88	1	154.88	21.29	0.0010*
$x_1\ x_3$	6.13	1	6.13	0.062	0.8083	$x_1\ x_3$	1.28	1	1.28	0.18	0.6837
$x_2\ x_3$	528.13	1	528.13	5.35	0.0433*	$x_2\ x_3$	228.98	1	228.98	31.48	0.0002*
Lack-of-fit	601.84	5	120.37	1.56	0.3183	Lack-of-fit	15.41	5	3.08	0.27	0.9122
Pure error	385.33	5	77.07			Pure error	57.33	5	11.47		
R^2 = 0.9223, adjusted R^2 = 0.8524, adequate precision = 12.342						R^2 = 0.9726, adjusted R^2 = 0.9479, adequate precision = 21.933					
<u>Alum</u>											
Model	6101.03	9	677.89	71.38	< 0.0001*	Model	3499.17	9	388.80	1029.17	< 0.0001*
y_1	3687.44	1	3687.44	388.27	< 0.0001*	y_1	2372.41	1	2372.41	6279.92	< 0.0001*

y_2	665.27	1	665.27	70.05	$< 0.0001^*$	y_2	382.47	1	382.47	1012.41	$< 0.0001^*$
y_3	1021.91	1	1021.91	107.60	$< 0.0001^*$	y_3	554.22	1	554.22	1467.06	$< 0.0001^*$
y_1^2	266.52	1	266.52	28.06	0.0003^*	y_1^2	46.91	1	46.91	124.18	$< 0.0001^*$
y_2^2	120.05	1	120.05	12.64	0.0052^*	y_2^2	2.19	1	2.19	5.80	0.0367^*
y_3^2	312.15	1	312.15	32.87	0.0002^*	y_3^2	90.89	1	90.89	240.60	$< 0.0001^*$
$y_1\ y_2$	2.00	1	2.00	0.21	0.6561	$y_1\ y_2$	0.000	1	0.000	0.000	1.0000
$y_1\ y_3$	84.85	1	84.85	8.90	0.0137^*	$y_1\ y_3$	32.00	1	32.00	84.71	$< 0.0001^*$
$y_2\ y_3$	50.00	1	50.00	5.26	0.0447^*	$y_2\ y_3$	32.00	1	32.00	84.71	$< 0.0001^*$
Lack-of-fit	64.14	5	12.83	2.08	0.2203	Lack-of-fit	0.94	5	0.19	0.33	0.8734
Pure error	30.83	5	6.17			Pure error	2.83	5	0.57		
R^2 = 0.9847, adjusted R^2 = 0.9709, adequate precision = 29.656						R^2 = 0.9989, adjusted R^2 = 0.9980, adequate precision = 116.451					
C. obtusifolia seed gum and alum											
Model	4058.49	9	450.94	80.94	$< 0.0001^*$	Model	3195.77	9	355.09	49.71	$< 0.0001^*$
z_1	637.64	1	637.64	114.46	$< 0.0001^*$	z_1	571.14	1	571.14	79.96	$< 0.0001^*$
z_2	620.37	1	620.37	111.35	$< 0.0001^*$	z_2	407.89	1	407.89	57.10	$< 0.0001^*$
z_3	1624.62	1	1624.62	291.62	$< 0.0001^*$	z_3	880.87	1	880.87	123.32	$< 0.0001^*$
z_1^2	28.84	1	28.84	5.18	0.0462^*	z_1^2	51.65	1	51.65	7.23	0.0227^*
z_2^2	28.84	1	28.84	5.18	0.0462^*	z_2^2	212.25	1	212.25	29.71	0.0003^*
z_3^2	953.03	1	953.03	171.07	$< 0.0001^*$	z_3^2	1158.06	1	1158.06	162.13	$< 0.0001^*$
$z_1\ z_2$	8.00	1	8.00	1.44	0.2584	$z_1\ z_2$	6.13	1	6.13	0.86	0.3762
$z_1\ z_3$	60.50	1	60.50	10.86	0.0081^*	$z_1\ z_3$	1.13	1	1.13	0.16	0.6998
$z_2\ z_3$	144.50	1	144.50	25.94	0.0005^*	$z_2\ z_3$	36.13	1	36.13	5.06	0.0483^*
Lack-of-fit	26.88	5	5.38	0.93	0.5298	Lack-of-fit	22.60	5	4.52	0.46	0.7912
Pure error	28.83	5	5.77			Pure error	48.83	5	9.77		
R^2 = 0.9865, adjusted R^2 = 0.9743, adequate precision = 30.516						R^2 = 0.9781, adjusted R^2 = 0.9585, adequate precision = 23.485					

*significant terms where p values < 0.05

Table 3

Reduced quadratic models using Design-Expert® in treatment of raw PPME.

<u>*C. obtusifolia* seed gum</u>
TSS removal = $-69.67 + 71.65X_1 + 10.33X_2 + 25.08X_3 - 7.36X_1^2 - 0.67X_3^2 - 9.67X_1X_2 - 3.50X_2X_3$
COD removal = $4.07 + 32.35X_1 - 16.49X_2 + 10.98X_3 - 2.67X_1^2 + 3.33X_2^2 - 5.24X_1X_2 - 2.31X_2X_3$
<u>Alum</u>
TSS removal = $-62.40 + 411.95Y_1 + 19.14Y_2 + 22.38Y_3 - 542.92Y_1^2 - 1.28Y_2^2 - 1.61Y_3^2 - 21.48Y_1Y_3 - 0.98Y_2Y_3$
COD removal = $-42.16 + 256.08Y_1 + 7.44Y_2 + 14.47Y_3 - 227.78Y_1^2 - 0.17Y_2^2 - 0.87Y_3^2 - 13.22Y_1Y_3 - 0.78Y_2Y_3$
<u>*C. obtusifolia* seed gum and alum</u>
TSS removal = $22.73 - 40.73Z_1 + 644.82Z_2 + 24.14Z_3 - 128.30Z_1^2 - 1471.96Z_2^2 - 2.81Z_3^2 - 15.41Z_1Z_3 - 80.65Z_2Z_3$
COD removal = $-21.88 + 32.86Z_1 + 800.44Z_2 + 25.88Z_3 - 171.72Z_1^2 - 3993.48Z_2^2 - 40.32Z_2Z_3$

Table 4

Optimal treatment conditions of raw PPME using *C. obtusifolia* seed gum and/or alum.

Coagulant	*C. obtusifolia* seed gum dosage, g/L	Alum dosage, g/L	Initial pH	Slow-mixing time, min	TSS removal, %			COD removal, %		
					Predicted	Experimental	Error	Predicted	Experimental	Error
C. obtusifolia seed gum alone	1.79	-	3.0	7.9	87.3	87.3±1.2	0	37.9	40.2±0.7	6.1
Alum alone	-	0.24	6.0	3.6	93.7	93.1±1.0	0.6	57.0	56.8±1.0	0.4
Combination between *C. obtusifolia* seed gum and alum*	0.17	0.09	Natural	3.4	91.6	89.6±0.5	2.2	58.3	55.4±0.5	5.0
Reduction of coagulant dosage, %	90.5	62.5								

*The coagulation process was done at natural pH of raw PPME, which was pH 7.2

Table 5

Market prices of *C. obtusifolia* seed gum and alum and treatment cost incurred for the present study.

Coagulant	Material cost (USD/metric ton)	Coagulant dosage (g/L)	Treatment cost (USD/L)
C. obtusifolia seed gum alone	400	1.79	7.2×10^{-4}
Alum alone	1000	0.24	2.4×10^{-4}
C. obtusifolia seed gum and	400	0.17	1.6×10^{-4}
alum	1000	0.09	